PTO 03-5299

CY=WO DATE=19940707 KIND=A1
PN=94-14465

STABLE LYOPHILIZED PHARMACEUTICAL PREPARATIONS OF G-CSF
[Stabile lyophilisierte pharmazeutische Zubereitungen von G-CSF]

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UNITED STATES PATENT AND TRADEMARK OFFICE
Washington, D.C. September 2003

Translated by: FLS, Inc.

PUBLICATION COUNTRY	(10): WO
DOCUMENT NUMBER	(11): 94/144465
DOCUMENT KIND	(12): A1 (13): Application
PUBLICATION DATE	(43): 19940707
PUBLICATION DATE	(45):
APPLICATION NUMBER	(21): PCT/EP93/03543
APPLICATION DATE	(22): 19931215
ADDITION TO	(61):
INTERNATIONAL CLASSIFICATION	(51): A61K 37/02
DOMESTIC CLASSIFICATION	(52):
PRIORITY COUNTRY	(33): DE
PRIORITY NUMBER	(31): P 4242863.7
PRIORITY DATE	(32): 19921218
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TITLE	(54): STABLE LYOPHILIZED PHARMACEUTICAL PREPARATIONS OF G-CSF
FOREIGN TITLE	[54A]: Stabile lyophilisierte pharmazeutische Zubereitungen von G-CSF

This invention relates to lyophilized pharmaceutical preparations of G-CSF that contain maltose, raffinose, sucrose, trehalose, or amino sugars as stabilizers. The invention also relates to a process for producing these stabilized lyophilizates and the use of maltose, raffinose, sucrose, trehalose, or amino sugars as stabilizers of G-CSF-containing medicines. /1*

Various pharmaceutical preparations containing G-CSF (granulocyte colony-stimulating factor) are already known from the prior art.

DE 37 23 781 (GB 2,193,631) describes a G-CSF-containing pharmaceutical that contains at least one pharmaceutical surface-active agent, saccharide, protein, or high-molecular compound to stabilize the G-CSF. It proposes preparations that contain human serum albumin as a stabilizing agent. Particularly advantageous preparations that are mentioned are preparations that contain surface-active agents, measured in parts by weight, corresponding to 1 to 10,000 times the quantity of G-CSF that is used.

EP 0 373 679 describes stabilized G-CSF preparations that are characterized, in particular, by an acidic pH value in the solution, whereby the solutions are intended to have as low a conductivity as possible. The solutions has a pH value of 3-3.7 if they contain additional pharmaceutical additives, such as buffer or mannitol. If

*Numbers in the margin indicate pagination in the foreign text.

no bufferr is present in the pharmaceutical dosage form, then pH values in the range of 2.75 to 4 are describes as being advantageous.

Moreover, EP 0 306 824 describes stabilized lyophilizates of human protein preparations, in which stabilization is achieved by adding a mixture of urea, amino acids, and detergent.

In the earlier PCT patent application PCT/EP92/01823, a process /2 is described for producing G-CSF-containing, well-tolerated pharmaceuticals for infusion or injection purposes. The liquid forms of administration are distinguished, in particular, by a low titration acidity and low buffer capacity. The pH value of the G-CSF-containing infusion and injection solutions described are in the acidic range of ca. 3.8-4.5.

A process for producing G-CSF-containing liquid pharmaceuticals that also contain preservatives is known from PCT/EP92/01822. The pH values of the aqueous pharmaceutical solutions are in the acidic range of 2.5-4.5. Here, the G-CSF is stabilized essentially by adjusting to acidic pH values that are favorable for G-CSF and by adding a mixture of various amino acids.

However, the previously known dosage forms for G-CSF have several disadvantages. It was found that in some cases liquid G-CSF preparations can be sensitive to freezing and thawing. Uncontrolled freezing and rethawing of such preparations can result in the appearance of dimers, oligomers, and aggregates and insoluble precipitates may be formed. Such properties of protein

pharmaceuticals are questionable from a medical and pharmaceutical standpoint since accidental freezing of the pharmaceutical solution cannot be avoided with certainty, so that there is a risk that a qualitatively altered preparation will be administered.

A disadvantage of the preparations described in De 37 23 781 is the fact that they pharmaceutical additives or auxiliary agents that cannot automatically be classified as harmless from a medical standpoint. With regard to their suitability as pharmaceutical additives, due to their origin and physical/chemical properties, polymers and proteins possess a certain risk potential. Proteins of human and animal origin and proteins taken from cell cultures have a potential residual risk of viral contamination. Due to their /3 antigen properties, other protein-like impurities that are analytically difficult to detect can cause immunological reactions in humans. Moreover, proteins of animal origin in general can trigger immunological reactions in humans, because of their species-specific properties. Long-term reactions following later reapplication of such proteins are also possible.

Adding high-molecular compounds (polymers) can also be problematic. Due to their high molecular mass, polymers can accumulate in the body and, if no biodegradation occurs, they can remain in the body for a long time. This is particularly dangerous with subcutaneous application, since removal and distribution through the bloodstream are much slower than with intravenous administration.

Depending on their molecular mass, polymers can also have antigen properties. It is also difficult to guarantee the purity of polymers, due to the catalysts used to produce them or to the presence of monomers and other polymer fragments. Consequently, the use of polymers in pharmaceutical forms of administration is to be avoided when possible, particularly for subcutaneous forms of administration.

The surfactant quantities described in DE 37 23 781 must also be considered problematic from a medical standpoint. Surfactant concentrations in parts by weight that are 1 to 10,000 times that of G-CSF are described there as advantageous. However, if we consider the concentrations of G-CSF administered in clinical use of 0.05 to 1.5 mg/ml in the final form of administration, then correspondingly high surfactant concentrations result. These should be avoided from a medical standpoint, since local irritation can result.

Moreover, several known formulations have the disadvantage that, because of the low pH values that are used, they can result in local intolerance by the patient, particularly in subcutaneous application. The product that is obtained can result in pain and local tissue irritation in sensitive patients, since they are not in the pH range of 7.0 to 7.5 found in physiological tissue. /4

It is also known from the literature that nonglycosylated forms of G-CSF, in particular, are particularly unstable, compared to glycosylated G-CSF obtained from CHO cells (J. Biol. Chem. 1990, 265, 11,432). The stabilization of nonglycosylated forms of G-CSF has

proven particularly difficult and requires special measures in order to formulate this molecule in a stable form of administration.

The object of this invention was to provide a form of administration of G-CSF that would make possible proper use of G-CSF as a medication, without the above-mentioned disadvantages found in earlier medications. The pharmaceutical preparation should be stable with respect to uncontrolled freezing and thawing and stable during long-term storage as a lyophilizate and it should be well-tolerated physiologically, easy to use, and available in precise doses.

The examples described in De 37 23 781 show that stable lyophilizates can be obtianed if human serum albumin is used as an auxiliary agent. The addition of sugar alcohols alone results in less stable formulations. Thus, in order to improve the state of the art, it is desirable to discover formulations that contain no human serum albumin (HSA) or other protein or polymer, yet possess good stability even at elevated temperatures. Renouncing the use of human serum albumin and polymers reduces the medical risk of side effects, as described for example for HSA.

Surprisingly, it was found that, in the sense of the present invention, stable pharmaceutical forms of administration can be produced if maltose, raffinose, sucrose, trehalose, or amino sugars are used as additives.

Solid preparations that contain maltose, raffinose, sucrose, /5
trehalose, or amino sugars as additives can be frozen or stored at elevated temperatures (up to 40°C) without significant loss of the quality of the proteins. The pharmaceutical quality of the active ingredient is not negatively affected by this. The preparations made in accordance with this invention are preferably made commercially available in the form of lyophilizates. Aqueous preparations made by redissolving are very well tolerated and are preparations of high quality, with regard to their protein stability. Moreover, they have the advantage that, due to the addition of maltose, raffinose, sucrose, trehalose, or amino sugars as additives, solutions can be produced with an advantageous pH value of 4-5 or 7-8, while for reasons of protein stability the solutions made by the prior art mainly require a pH of 2.5-3.5.

The preparations made in accordance with this invention also have the advantage of being essentially free of protein-like or polymer additives, whose use can be problematic from a medical standpoint. Since liquid G-CSF-containing forms of administration with a pH of ca. 4-5 or 7-8, preferably with a pH in the vicinity of the pH value of the blood (pH 7.2-7.4) can now be produced by dissolving lyophilizates, they also have the advantage of being well tolerable and generally pain free in administration. This is essential, in particular, for subcutaneous application, since in this case intolerance can occur more easily than with intravenous

administration. The preparations made in accordance with this invention can also be produced in concentrations of 0.005-1.5 mg/ml which are favorable clinically, so that injection volumes of ≤ 1.0 ml can be used. Small injection volumes are particularly advantageous with subcutaneous administration, since they cause only slight mechanical irritation.

It is also advantageous that, because of the additives selected, the previously required high surfactant quantities in the liquid forms are no longer needed. Moreover, low surfactant quantities of 0.5 mg/ml or less, preferably 0.01-0.1 mg/ml, are sufficient for /6 stabilizing G-CSF. Also advantageous is the fact that surfactant concentrations (mg/ml) can be used that are less than or, at a maximu, equal to the amount of G-CSF protein used per unit volume (mg/ml). This is advantageous, in particular, with liquid forms of administration that are designed for subcutaneous use of G-CSF. Moreover, by using the measures described in this invention, the labile, nonglycosylated G-CSF molecule can be stabilized sufficiently for pharmaceutical preparations.

The additive maltose (malt sugar, maltobiose, 4-O-alpha-D-glucopyranosyl-D-glucose) is used at an amount of 0.01-10,000 times the amount of the active ingredient G-CSF. The same applies to the additives raffinose, sucrose, and trehalose. The concentration of these substances in the liquid form of administration is 0.1-200 mg/ml, preferably 10-60 mg/ml. Instead of maltose, it is also

possible to use the stereoisomeric disaccharides cellobiose, gentiobiose, or isomaltose. In general, amino sugars refer to monosaccharides that have an amino or an acylated amino group instead of a hydroxy group. Examples include glucosamine, galactosamine, and neuraminic acid.

In a particular embodiment, pharmaceutical preparations are provided that contain amino acids, in addition to maltose, raffinose, sucrose, or trehalose. Possible amino acids include basic amino acids, such as arginine, lysine, ornithine, etc., acidic amino acids, such as glutamic acid, aspartic acic, etc., or aromatic amino acids, such as phenylalanine, tyrosine, tryptophane, etc.

Amino acids are used in amounts of 0.01 to 10,000 times the amount of G-CSF. The concentration of this additive in the liquid form of administration is 0.1-200 mg/ml, preferably 1-50 mg/ml.

To produce the lyophilizates, the aqueous pharmaceutical solutions are first produced, containing the active ingredient and other common pharmaceutical additives. Possible pharmaceutical additives include, in particular, amino acids, such as arginine, lysine, ornithine, phenylalanine, or tyrosine. Moreover, the aqueous preparations can also contain common buffer substances, such as acetic acid, hydrochloric acid, citric acid, lactic acid, tartaric acid, maleic acid, and phosphoric acid or their physiologically tolerated salts. For producing the additive solution, these buffer substance can be in the form of the corresponding free acid or the

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alkali, alkaline-earth, or ammonium salts. Moreover, the solution can contain additional common pharmaceutical additives.

The sequence in which the various additives or active ingredient are added is generally independent of the production process and is at the discretion of those skilled in the art. The desired pH value of the solution is adjusted by adding bases, such as alkali hydroxides, alkaline-earth hydroxides, or ammonium hydroxide. Preferably, sodium hydroxide is used. Adjustment to the desired pH is possible in principle by adding basic solutions. In this context, salts of strong bases with weak acids are considered, for example sodium acetate, sodium citrate, disodium or dipotassium hydrogen phosphate, or sodium carbonate. If the pharmaceutical additive solution has a basic pH value, the adjustment is made by titration with acid, until the desired pH region is reached. Possible acids include physiologically tolerated inorganic and organic acids, such as hydrochloric acid, phosphoric acid, acetic acid, citric acid, or the usual solutions of substances that posses an acidic pH value. Preferred substances in this context are salts of strong acids with weak bases, such as sodium dihydrogen phosphate or potassium dihydrogen phosphate.

The concentrations of buffer substances in the finished liquid form of administration are ca. 2-80 mol/liter. The overall concentration of buffer substances should not exceed 100 mmol/liter.

Preferably, the concentrations of buffer substances are 5-40 mmol/liter.

Stabilization of G-CSF molecules with the above-mentioned additives applies, in general, to all G-CSF molecules produced by recombinant processes and their variations. The term G-CSF or G-CSF variants in the present invention includes all naturally existing variants of G-CSF and modified G-CSF proteins derived from them by recombinant DNA technology, particularly fusion proteins, which contain other protein sequences in addition to the G-CSF portion. Particularly preferred in this context is G-CSF mutein with an N-terminal met residue at position -1, which is suitable for expression in prokaryotic cells. Also suitable is a recombinant methionine-free G-CSF variant that can be produced in accordance with PCT/EP91/00192. G-CSF variant refers to G-CSF molecules in which one or more amino acids can be deleted or replaced with other amino acids, whereby the essential characteristics of G-CSF are by and large retained. Suitable G-CSF muteins are described, for example, in EPO 456 200. /8

For producing well tolerated parenteral forms of administration, the addition of isotonic substances is useful, if isotonicity cannot be achieved by the osmotic properties of the active ingredient and the additives that are used for stabilization. Nonionized, well tolerated additives are primarily used.

The addition of salts to adjust the isotonicity is not advantageous, since high salt or ion concentrations promote aggregate

formation in G-CSF. Thus, it is advantageous to add salts in smaller quantities.

In addition, the pharmaceutical preparations can contain additional common auxiliary or additive agents. Antioxidants such as glutathione or ascorbic acid or similar substances, chaotropic additives such as urea, and amino acids such as arginine, lysine, ornithine, glutamic acid, and others can be used.

Below, the invention will be described in greater detail with /9 the help of representative examples.

Examples 1-14 show the manner in which lyophilizates can be formulated, produced, and examined with regard to protein stability, in accordance with this invention. The influence of the additives that are added in addition to maltose, raffinose, sucrose, or trehalose and of the pH is examined.

Comparative investigations of lyophilizates based on mannitol or glycine show that maltose, raffinose, sucrose, or trehalose lyophilizates produce significantly better results than preparations produced with other builders. Using the lyophilizates described in this invention and explained in the examples, produces an optimal formulation, with respect to the object described above, that has a tolerable pH value, long-term storage stability and that is able to withstand elevated storage temperatures and mechanical stress without negative effects on the protein. In particular, the preparations are insensitive to freezing and there is no reason to use additives that

are considered critical, such as proteins and polymers. Moreover, they contain only small quantities of physiologically well-tolerated surfactants.

Example 3 examines various sugars and sugar alcohols for their stabilizing effect in G-CSF lyophilizates. Maltose proved to be advantageous, compared to lactose and mannitol.

Example 4 describes lyophilizates with maltose and other additives. The results make clear that the addition of a surfactant does not significantly influence the stability of the preparation, although it prevents the adhesion of protein to surfaces, thereby preventing a possible loss of content. Thus, the presence of a surfactant is required in such formulations not for the purpose of stabilization, but for maintaining the nominal dosage.

Example 5 compares various maltose-containing lyophilizate /10 formulations with two otherwise identically formulated lyophilizates without maltose. It may be clearly seen from the data that the presence of maltose advantageously influences the parameters examined, with regard to the stability of the preparation. The addition of other additives, such as ascorbic acid, glutathione, or glutamic acid, at the storage temperatures and times examined, has no significant influence on stability. The preparations described in example 5 are characterized by the fact that in long-term storage at elevated temperature they show no change in the quality characteristics that were examined.

It may also be seen from the examples that in lyophilizates that contain maltose and arginine no additional buffer salt is absolutely needed, since the arginine buffer produced during pH adjustment with hydrochloric acid, phosphoric acid, citric acid, or other acid has a sufficient pH-stabilizing effect. Arginine buffer is excellently suitable for formulating preparations in the pH range below 5.0 and from 7.0 to 7.5 (see examples 11 and 12). Example 9 shows that redissolved lyophilizates with pH 7.4 that contain maltose and arginine buffer may be stored for at least 24 hours.

Example 6 describes G-CSF lyophilizates that contain amino sugars (galactosamine, N-methylglucosamine). It may be seen that the combination of maltose and amino sugar produces more stable preparations than the combination of glycine and amino sugars. This demonstrates that maltose in combination with physiologically well tolerated additives produces clearly more stable lyophilizates of G-CSF and, thus, lyophilizates of greater pharmaceutical quality than other builders and stabilizers proposed in the literature.

Example 7 shows that G-CSF in maltose-containing lyophilizates is clearly more stable than mannitol-containing lyophilizates. This is substantiated at the relevant storage temperatures and long storage times.

Example 8 shows that maltose-containing lyophilizates at various pH values and with various additives produce advantageous results, compared to lyophilizates with other builders and stabilizers (sugar alcohols, amino acids). /11

Example 10 demonstrates the stability of the lyophilizates with maltose, raffinose, sucrose, or trehalose, made in accordance with this invention, after storage at 40°C for 13 weeks.

Example 11 shows that the lyophilizates made in accordance with this invention are stable even with elevated G-CSF concentrations and example 12 demonstrates the long-term stability of the formulations made in accordance with this invention, even at elevated temperatures.

Example 1:

Experimental methods for stabilization determination

The lyophilized preparations were stored in the dark at specified storage temperatures and then studied by reversed phase HPLC (RP-HPLC), gel chromatography, or size exclusion chromatography (SEC HPLC), and western blot for their protein purity and for the appearance of aggregates and dimers. Moreover, the protein content was studied by OD 280 photometry, biological activity by bioassay (NFS 60 cell test), and aggregation and precipitation by turbidity measurements. The methods that were used can be described as follows:

1.1 **Reversed phase HPLC**

RP-HPLC was carried out using a Nucleosil C18 column (Knauer). The mobile phase comprised 0.12% (v/v) trifluoroacetic acid (TFA)/water (A) and 0.1% (v/v) TFA/acetonitrile (B). The /12 chromatography was carried out at a flow rate of 0.5 ml/min, using a linear gradient of A to B.

The injection quantity was 3-6 µg G-CSF, depending on the formulation. The peak area was evaluated using an external standard at a wavelength of 214 nm.

1.2 **Size exclusion chromatography (SEC)**

A TSK G 2000 SW separation column (7.5 × 300 mm) was used for the SE chromatography. The separations were made isocratically at room temperature and a flow rate of 0.6 ml/min in a phosphate buffer (22.2 mM Na_2HPO_4; 107.7 mM KH_2PO_4; pH 6.2). The injection quantity was 3-6 µg G-CSF. The peak area was evaluated using an external standard at a detection wavelength of 214 nm

1.3 **SDS page/western blot**

3 µg rhG-CSF is applied to a 12% polyacrylamide SDS gel under nonreducing conditions and subjected to gel electrophoresis. Then the G-CSF monomers, dimers, or aggregates, separated in

accordance with their molecular weight, are transferred to nitrocellulose by electroblotting. By incubation with a specific polyclonal biotinylated anti-G-CSF antibody (PAK<G-CSF>IgG) the protein bands are identified and detected by the phosphatase technique using streptavidin-alkaline phosphatase conjugate (SA-AP conjugate), 5-bromo-4-chloro-3-indolyl phosphate (BCIP) and nitro blue tetrazolium (NTB). The percentages of monomers, dimers, and aggregates were determined by laser densitometry, with the help of a rhG-CSF standard series.

1.4 NFS-60 bioassay (biological activity) /13

The in vitro activity determination of G-CSF is based on measuring the cell numbers in a cell culture of NFS-60 cells stimulated by G-CSF.

Under suitable conditions the dehydrogenase activity of the cells can be correlated with the G-CSF concentration in the medium. Suitable dilutions of the G-CSF buffer solution are produced, in order to obtain a simply measurable increase in dehydrogenase activity.

The activity is measured photometrically at 570 and 690 nm. The reduction of the tetrazolium salt MTT (yellow) to formazan (blue) is measured.

The in vitro activity of G-CSF is calculated by comparing the data of the sample to the standard, using the parallel-line method. The assessment is made in accordance with the requirements of Ph. Eur. (VIII, 13).

1.5 Light-scattering measurement, turbidity measurement

The measurement is made directly on the undiluted product solution in glass cuvettes (2 cm diameter). The diffusely deflected scattered light from the liquid is measured at an angle of 90°C. The measurement is compared to standard formazin suspensions in accordance with DIN 38404C2. The values are indicated in TE/F. The measurement is made with a suitable turbidity photometer, such as an LTP 5 (Dr. Lange, Dusseldorf).

1.6 Photometry OD 280 (protein content) /14

The G-CSF UV spectrum has an absorption maximum at 280 nm that is linked to side-chain chromophores such as residues of tryptophane, tyrosine, and phenylalanine. The measurement is compared to placebo solutions by

- a UV spectrophotometer
  (e.g. Uvikon 810 P or 941, Kontron Instruments)

- Semimicro quartz cuvettes, 500 µl, layer thickness:

1 cm

(e.g. Hellma, Suprasil, Cat. NO. 104.002B-QS)

Example 2:

Aqueous solutions of 0.1 mg/1 ml poloxamer 118 and 50 mg/ml of the sugar or sugar alcohols indicated--Mannitol (formulation 1), lactose (formulation 2), and maltose (formulation 3)--were treated with G-CSF at a concentration of 70 µg/ml. After filtration through a sterilized 0.2 µm membrane filter, the solutions were filled into sterile injection flasks made of glass of hydrolytic class I. After lyophilization, they were aerated with sterile nitrogen and the initially loose stoppers were pressed in under aseptic conditions to seal the lyophilizates. The lyophilizates were flanged and kept in the dark at various temperatures for 6 and 13 weeks. Then the stability of the preparations was studied using the methods described below.

Table 1a: Storage at 20°C /15

	Storage 6 weeks at 20°C			Storage 13 weeks at 20°C		
	I	II	III	I	II	III
Formulation 1, mannitol	>99%	91%	36%	86%	47%	27%
Formulation 2, lactose	>99%	>99%	18%	>99%	>99%	12%
Formulation 3, maltose	>99%	>99%	6%	>99%	>99%	7.8%

I Purity, unchanged protein in RP HPLC
II Purity, unchanged protein in SEC HPLC
III Dimers/aggregates in western blot

Table 1b: Storage at 40°C

	Storage 6 weeks at 40°C			Storage 13 weeks at 40°C		
	I	II	III	I	II	III
Formulation 1, mannitol	81%	70%	50%	69%	25%	70%
Formulation 2, lactose	>99%	>99%	37%	>99%	>99%	Not detectable/aggregated
Formulation 3, maltose	>99%	>99%		>99%	>99%	12%

I Purity, unchanged protein in RP HPLC
II Purity, unchanged protein in SEC HPLC
III Dimers/aggregates in western blot

Example 3 /16

Lyophilizates of G-CSF were produced. For this purpose, the auxiliary agents indicated in the table below were dissolved in water for injection, after which G-CSF was added at a concentration of 70 µg/ml and, optionally, the pH was precisely adjusted using small quantities of the buffer system. Pluronic F 68 was used to represent an appropriate surfactant. Other surfactants behave similarly. Following sterile filtration through a suitable 0.2 µm membrane filter, the solutions were filled into sterile injection flasks made of glass of hydrolytic class I and lyophilized by the usual methods. After lyophilization, they were aerated with nitrogen and the injection flasks were sealed with freeze-dried stoppers under aseptic

conditions. The preparations were stored in flanged bottles and kept in the dark at specified storage temperatures for 6 and 12 weeks and studied using the methods mentioned in example 1.

Table 2: Maltose-containing formulations of G-CSF at pH 3.6

	Formulation 4	Formulation 5
G-CSF	70µg	70µg
Maltose	35mg	35mg
L-Phenylalanine	10mg	10mg
Ascorbic acid	5mg	5mg
Glutathione	10mg	10mg
L-Glutamic acid	5mg	5mg
L-Arginine	10mg	10mg
Buffer (pH)	ad pH 3.6	ad pH 3.6
Pluronic F68	-	0.1mg
Water for injection	ad 1 ml	ad 1 ml

Table 3: Storage at 20°C /17

Formulation	Storage temperature	After 6 weeks; Aggregate in %	After 12 weeks; Aggregate in %	After 6 weeks; SEC, %G-CSF; RP, %G-CSF		After 12 weeks; SEC, %G-CSF; RP, %G-CSF	
4	+20°C	0.0	2.6	61	63	64	69
5	+20°C	0.0	0.5	>99	>99	>99	>99

Example 4:

G-CSF lyophilizates with 500 mg G-CSF (formulations 6-10) were prepared as follows. The auxiliary agents indicated in the table below were dissolved in water for injection, G-CSF was added and, if

necessary, the pH was adjusted with small amounts of hydrochloric acid or disodium hydrogen phosphate. In each case, 1 ml of the solutions, previously sterile filtered through a 0.2 µm membrane filter, were filled into injection flasks made of glass of hydrolytic class I and freeze dried by the usual methods. After lyophilization, they were aerated with nitrogen and the lyophilizates were sealed with lyophilization stoppers under aseptic conditions. The flanged lyophilizates were stored in the dark at specified temperatures and examined by the methods mentioned in example 1.

Table 4: Composition of formulations 6-10

	Formulation 6	Formulation 7	Formulation 8	Formulation 9	Formulation 10
G-CSF	0.5µg	0.5µg	0.5µg	0.5µg	0.5µg
Maltose	35mg	35mg	35mg	-	-
L-Phenylalanine	10mg	10mg	10mg	10mg	10mg
Ascorbic acid	5mg	-	-	5mg	-
Glutamin	10mg	-	-	10mg	-
L-Glutaminsaure	5mg	-	-	5mg	-
L-Arginin	10mg	10mg	10mg	10mg	10mg
Puffer (pH)	ad 4.5	ad 4.5	ad 6.5	ad 4.5	ad 6.5
Pluronic F68	0.1mg	0.1mg	0.1mg	0.1mg	0.1mg
Water for injection	ad 1 ml	ad 1 ml	ad 1 ml	ad 1 ml	ad 1 ml

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Table 5: Results of analysis

(a) (b) (c) (d) (e)

R z.	Lag r-temp.	Western Blot 6 Wo. % Aggr.	12 Wo. % Aggr.	nach 6 Wochen SEC % G-CSF	% Aggr.	RP % G-CSF	nach 13 Wochen SEC % G-CSF	% Aggr.	RP % G-CSF
6	+ 8 °C	< 1	1,0	> 99 %	0,9	> 99 %	> 99 %	0,7	99 %
	+ 40 °C	< 1	1,7	> 99 %	0,6	> 99 %	> 99 %	0,6	98 %
7	+ 8 °C	< 1	1,1	> 99 %	1,6	> 99 %	> 99 %	1,1	99 %
	+ 40 °C	< 1	2,3	> 99 %	1,9	> 99 %	> 99 %	1,1	99 %
8	+ 8 °C	< 1	-				> 98 %	1,5	> 99 %
	+ 40 °C	< 1	-				> 98 %	1,4	> 99 %
9	+ 8 °C	3,8	0,3	95 %	5,8	> 99 %	95 %	1,5	98 %
	+ 40 °C	7,9	2,5	95 %	6,4	93 %	86 %	0,8	94 %
10	+ 8 °C	-	5,2				96 %	1,0	95 %
	+ 40 °C	-	10,3				89 %	2,6	89 %

Key:
a) Formulation
b) Storage temperature
c) Western blot; 6 weeks, %aggregate; 12 weeks, %aggregate
d) After 6 weeks; SEC %G-CSF, %aggregate, RP, %G-CSF
e) After 13 weeks; SEC %G-CSF, %aggregate, RP, %G-CSF

Example 5: /19

The formulations given in the table below (formulations 11-14) were prepared as follows: the auxiliary agents were dissolved in water for injection, then G-CSF was added at the indicated concentrations. If necessary, the pH was precisely adjusted using the components of the phosphate buffer. The solutions were then filtered through a sterilized membrane filter with a pore width of 0.2 □m and filled into sterile injection flasks made of glass of hydrolytic

class I and lyophilized. After lyophilization they were aerated with nitrogen, the lyophilizates were sealed with lyophilization stoppers under aseptic conditions and flanged. The lyophilizates were stored at specified temperatures. After 6 and 13 weeks, studies were conducted using the methods indicated in example 1.

Table 6: Amino sugar-containing lyophilizate preparations

	(a)	(b)	(c)	(d)
	Rez. 11	**Rez. 12**	**Rez. 13**	**Rez. 14**
(e) **G-CSF**	0,5 µg	0,5 µg	0,5 µg	0,5 µg
(f) **Pluronic F68**	0,1 mg	0,1 mg	0,1 mg	0,1 mg
(g) **N-methyl-glucosamin**	-	10 mg	-	10 mg
(h) **Galactosamin**	10 mg	-	10 mg	-
(i) **Glycin**	-	-	35 mg	35 mg
(j) **Maltose**	35 mg	35 mg	-	-
(k) **Phenylalanin**	10 mg	-	-	-
(l) **Phosphatpuffer**	ad pH 7,0	ad pH 7,0	ad pH 7,0	ad pH 7,0
(m) **Wasser für Injektionszwecke**	ad 1,0 ml	ad 1,0 ml	ad 1,0 ml	ad 1,0 ml

Key:
a) Formulation 11
b) Formulation 12
c) Formulation 13
d) Formulation 14
e) G-CSF
f) Pluronic F68
g) N-methyl glucosamine
h) Galactosamine
i) Glycine
j) Maltose
k) Phenylalanine
l) Phosphate buffer
m) Water for injection

The analytical data obtained after storage of the above-mentioned preparations are summarized in the following table of results.

Table 7: Results of analysis /20
DCP = decomposition products

(a) (b) (c) (d) (e) (f) (g)

Rez.	Lagerung	6 Wochen West. Blot. % Aggr.	12 Wochen West. Blot. % Aggr.	12 Wochen RP-HPLC % G-CSF	12 Wochen % ZSP in SEC HPLC
11	+ 8 °C	3,8	2,9	> 99	1,2
	+ 40 °C	3,2	2,3	> 99	1,8
12	+ 8 °C	1,8	3,8	> 99	1,4
	+ 40 °C	1,7	4,5	> 99	0,7
13	+ 8 °C	1,1	1,4	> 99	0,9
	+ 40 °C	16,8	13,0	75	4,2
14	+ 8 °C	1,6	12,4	97,5	1,2
	+ 40 °C	7,7	26,3	84,5	3,5

Key:
a) Formulation
b) Storage
c) 6 weeks, Western blot; %Aggregate
d) 12 weeks, Western blot; %Aggregate
e) 12 weeks
f) RP-HPLC; %G-CSF
g) %DCP in SEC HPLC ,

Example 6:

Formulations 15 and 16 described below were prepared as follows: the auxiliary agents indicated were dissolved in water for injection and G-CSF was added at the indicated concentration. The pH was adjusted, if necessary, using portions of the buffer components. Then the solution was filtered through a sterilized membrane filter with a

pore width of 0.2 µm and filled under aseptic conditions into sterile injection flasks made of glass of hydrolytic class I. Then the injection preparations were freeze dried, they were aerated with nitrogen, and the injection flasks were sealed with lyophilization stoppers under aseptic conditions and flanged. The preparations were stored in the dark at specified temperatures and examined for the parameters indicated below. The study methods indicated in example 1 were used.

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Formulation 15

G-CSF
Polysorbate 80
Phenylalanine
Maltose
L-Arginine
Buffer
Water for injection

Formulation 16

G-CSF
Polysorbate 80
Mannitol
Buffer
Water for injection

Table 8: Study results after storage of formulations 15 and 16 for 3 and 6 months

	Formulation 15				Formulation 16			
	Storage time3 months		Storage time6 months		Storage time3 months		Storage time6 months	
	4-8°C	23°C	4-8°C	23°C	4-8°C	23°C	4-8°C	23°C
Western blot(dimers)	2.2	<1%	1.3%	0.7	<1%	12%	4.1%	17
SEC-HPLC(dimers)	<1%	<1%	<1%	<1%	<1%	2%	<1%	3
RP-HPLC(G-CSF peak)	>99%	>99%	>98%	>98	>99%	98.2%	>98%	>98

Example 7: /22

The formulations described in table 9 were prepared as follows:

The indicated auxiliary agents were dissolved in water for injection. G-CSF was added at the concentration indicated, after which the pH was adjusted with small portions of the buffer components, if necessary. The pharmaceutical solutions were then filtered through a sterilized membrane filter with a pore width of 0.2 □m, then filled under aseptic conditions into sterilized injection flasks made of glass of hydrolytic class I and lyophilized.

After lyophilization, they were aerated with nitrogen and the flasks were sealed under aseptic conditions with lyophilization stoppers. The flasks were flanged and stored in the dark under specified temperature conditions. After the specified storage time, the analytical tests described in example 1 were carried out (c.f. table 10).

Table 9: G-CSF lyophilizates with maltose, compared to other builders /23

	Rez. 17	Rez. 18	Rez. 19	Rez. 20	Rez. 21	Rez. 22	Rez. 23	Rez. 24
G-CSF	350 µg	350 µg	175 µg	175 µg	175 µg	175 µg	175 µg	175 µg
Maltose			17,5 mg	17,5 mg	17,5 mg	-	-	-
Glycin						4 mg	10 mg	8,9 mg
Arginin			5 mg	-	5 mg	5 mg	-	
Phenylalanin			5 mg	5 mg	5 mg	5 mg	-	2,5 mg
Mannit	50 mg	50 mg				-	-	
Tween 80	0,1 mg	0,1 mg	0,05 mg	0,05 mg	0,05 mg	0,05 mg	0,05 mg	0,05 mg
pH	4,5	7,2	4,5	7,2	7,2	7,2	7,2	7,2
(Puffer)	HCl	Phosphat	HCl	HCl	HCl	HCl	Phosphat	Phosphat
Wasser für Injektionszwecke	ad 1,0 ml	ad 1,0 ml	ad 0,5 ml	ad 0,5 ml	ad 0,5 ml	ad 0,5 ml	ad 0,5 ml	ad 0,5 ml

Key:
Rez.=Formulation
Arginin=Arginine
Phenylalanin=Phenylalanine
Mannit=Mannitol
(Puffer)=(Buffer)
Phosphat=Phosphate
Wasser für Injektionszwecke=Water for injection

Table 10 Summarizes the analytical data obtained for the formulations indicated: /24

Formulation	Storage	4 Weeks RP-HPLC %G-CSF	4 Weeks SEC-HPLC %G-CSF	4 Weeks Western blot
17	8 °C	> 99	> 99	3,6 % Dimere
	30 °C	94	92	9,6 % Dimere
	40 °C			14,4 % Dimere
18	8 °C	69	60	Aggregate
	30 °C	44	36	Aggregate
	40 °C	13	12	Aggregate
19	8 °C	> 99	> 99	1,0 % Dimere
	30 °C	> 99	95, 5	0,5 % Dimere
	40 °C	> 99	97,5	0,5 % Dimere
20	8 °C	> 99	> 99	1,6 % Dimere
	30 °C	> 99	> 99	1,4 % Dimere
	40 °C	> 99	> 99	2,3 % Dimere
21	8 °C	> 99	> 99	1,5 % Dimere
	30 °C	> 99	97,5	2,1 % Dimere
	40 °C	> 99	97	2,0 % Dimere
22	8 °C	> 99	> 99	2,8 % Di/Aggregat
	30 °C	96	96	3,0 % Di/Aggregat
	40 °C			12 % Di/Aggregate
23	8 °C	> 99	> 99	6,8 % Dimere
	30 °C	91,5	92	Aggregate
	40 °C	79	74	Aggregate
24	8 °C	> 99	> 99	10,8 % Dimere
	30 °C	88	85	Aggregate
	40 °C	67	60	Aggregate

Dimere=Dimers
Aggregate=Aggregates

Example 8: /25

Holding time of redissolved lyophilizat s made in accordance with this invention at pH 7.4

The following compositions were prepared:

mg/ml	Formulation 25
G-CSF	0.35
Polysorbate 80	0.1
Maltose	50
Arginine	10
Phenylalanine	10
Hydrochloric acid	ad pH 7.4

The auxiliary agents mentioned were dissolved in 1 ml water for injection. G-CSF was added, and the pH was adjusted to 7.4. The solution was filtered through a sterilized membrane filter with a pore width of 0.2 µm and then filled into injection flasks of hydrolytic class I.

After suitable lyophilization stoppers were inserted, the preparation was freeze dried at a main drying temperature of -25°C and a subsequent drying temperature of +8°C until a residual moisture content of <5% was achieved. The dried lyophilizates were aerated with nitrogen and sealed.

After storage for 6 months at 4-8°C, the lyophilizates were dissolved in water for injection and then allowed to stand at room temperature for 24 hours.

After this standing time, the examination methods described in example 1 for biological activity (NFS-60 test), protein content (photometry OD 280), purity (western blot), purity (SEC HPLC), /26 purity (SDS page), and purity (RP HPLC) showed no change, compared to samples examined immediately following dissolution. Turbidity measurements also revealed very low turbidity values, even under mechanical stress.

Thus, it is clear that redissolved lyophilizates made by the formulation of this invention with maltose and arginine buffer at pH 7.4 have a sufficient standing time for clinical use.

Example 9:

Stability of lyophilizates made in accordance with this invention containing maltose, raffinose, sucrose, or trehalose after storage for 13 weeks at 40°C

Three lyophilizates were prepared in accordance with example 8, formulation 25, containing 50 mg/ml maltose or an equal weight of a) raffinose or b) sucrose or c) trehalose.

All the lyophilizates were stored for 13 weeks at temperatures of 5, 25, 30, and 40°C, then dissolved and examined visually and using the examination methods SEC HPLC, RP HPLC, western blot, and SDS page, described in Example 1.

Clear colorless solutions were obtained in all cases. In SEC HPLC the size of the product peak was was >98% and that of the dimers/aggregates <1%. In RP HPLC the peaks reached 100%. Side peaks

were not detected. The main peak corresponding to the working standard. No decomposition products, dimers, or aggregates were detected in SDS page.

Table: after 13 weeks of storage /27

Temp.	SEC HPLC G-CSF	RP HPLC %G-CSF	Western blot %aggregate	SDS Page %side bands
5 °C	> 98 %	100 %	n.n.	< 1
20 °C	> 98 %	100 %	n.n.	< 1
30 °C	> 98 %	100 %	n.n.	< 1
40 °C	> 98 %	100 %	n.n.	< 1

Example 10:

Stability of maltose lyophilizates made in accordance with this invention with arginine phosphate and arginine chloride buffers at pH 4.5 and pH 7.2 after storage for 13 weeks at 30°C

Using the method described in example 8, the following formulations were prepared, which differ only in their buffers and pH values:

	R z ptur 26	R z ptur 27	Rezeptur 28	Rezeptur 29
G-CSF	0,35 mg	0,35 mg	0,35 mg	0,35 mg
Polysorbat 80	0,1 mg	0,1 mg	0,1 mg	0,1 mg
Phenylalanin	10 mg	10 mg	10 mg	10 mg
Arginin	10 mg	10 mg	10 mg	10 mg
Maltose	47,5 mg	47,5 mg	47,5 mg	47,5 mg
Phosphorsäure	ad pH 4,5	ad pH 7,2		
Salzsäure			ad pH 4,5	ad pH 7,2

Rezeptur=Formulation
Polysorbat=Polysorbate
Phenylalanin=Phenylalanine
Arginin=Arginine
Phosphorsäure=Phosphoric acid
Salzsäure=Hydrochloric acid

The preparations were stored at temperatures of 4 to 8°C, /28 20-25°C, and 30°C. After 13 weeks they were dissolved in 1 ml water for injection and examined by the methods described in example 1: RP HPLC, SEC HPLC (purity), and western blot (decomposition, dimerization, and aggregate formation). The results are presented in table 11 and they show that the lyophilizates made in accordance with this invention with pH 4.5 and 7.2 are stable even after 13 weeks of storage at 30°C.

Table 11: Results after 13 weeks at 30°C

	RP-HPLC	SEC HPLC	Western blot		
	%side peaks		Decomposition	Dimers	Aggregate
Rezeptur 26	< 1	< 1	n.n.	n.n.	n.n.
Rezeptur 27	< 1	< 1	n.n.	n.n.	n.n.
Rezeptur 28	< 1	< 1	n.n.	< 1 %	n.n.
Rezeptur 29	< 1	< 1	n.n.	< 1 %	n.n.

Rezeptur=Formulation
n.n.=not detectable

Example 11:

Stability of maltose lyophilizates made in accordance with this invention with arginine phosphate and arginine chloride buffer at pH 7.4 after 4 and 13 weeks of storage at 40°C

Formulations were prepared as in example 8 and their pH values were adjusted to 7.4, once with hydrochloric acid and once with phosphoric acid.

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	Rezeptur 30	Rezeptur 31
G-CSF	0,35 mg	0,35 mg
Polysorbat 80	0,1 mg	0,1 mg
Phenylalanin	10 mg	10 mg
Arginin	10 mg	10 mg
Maltose	47,5 mg	47,5 mg
Phosphorsäure	pH 7,4	
Salzsäure		pH 7,4

Rezeptur=Formulation
Polysorbat=Polysorbate

Phenylalanin=Phenylalanine
Arginin=Arginine
Phosphorsäure=Phosphoric acid
Salzsäure=Hydrochloric acid

These two preparations were stored at temperatures of 4 to 8°C and 40°C for 4 and 13 weeks. The results of the tests (western blot, SDS Page) after 13 weeks of storage are presented in Table 12 below. The results after 4 weeks of storage are identical.

The results show that the maltose lyophilizates made in accordance with this invention are stable at pH 7.4, even after being stored for 13 weeks at 40°C.

Table 12: Results after 13 weeks at 40°C

	(a) Lager temp.	(b) Western Blot, nicht reduz. (c) Aggr. < 1 %	(d) Dimere < 2 %	(e) SDS-Page, reduz. (f) Produkt-Bd.	(g) Abbau-prod.	(h) Zusatz-bd.	(i) Rest-feuchte %
Rezeptur 30	5 °C	n.n.	n.n.	< 98 %	< 1 %	n.n.	1,4
	40 °C	n.n.	n.n.	< 98 %	< 1 %	n.n.	2,0
Rezeptur 31	5 °C	n.n.	n.n.	< 98 %	< 1 %	n.n.	1,9
	40 °C	n.n.	n.n.	< 98 %	< 1 %	n.n.	2,1

Key to table 12:
a) Storage temperature
b) Western blot, non-reduc.
c) Aggregate
d) Dimers
e) SDS Page, reduc.
f) Product band
g) Decomposition products

h) Additional bands
i) Residual moisture
Rezeptur=Formulation
n.n.=not detectable

Example 12: /30

Stability of lyophilizates made in accordance with this invention at pH 7.4 and G-CSF concentrations of 0.5 and 1.0 mg/ml after storage for 13 weeks at 40°C

The following formulations, which differ in their G-CSF content, were prepared in accordance with the method described in example 8:

mg/ml	Rezeptur 32	Rezeptur 33
G-CSF	0,5 mg	1,0 mg
Polysorbat 80	0,1 mg	0,1 mg
Phenylalanin	10 mg	10 mg
Arginin	10 mg	10 mg
Maltose	47,5 mg	47,5 mg
Phosphorsäure	pH 7,4	pH 7,4

Rezeptur=Formulation
Polysorbat=Polysorbate
Phenylalanin=Phenylalanine
Arginin=Arginine
Phosphorsäure=Phosphoric acid

The preparations were stored at -20°C, 4-8°C, 20-25°C, 30°C, and 40°C for 4 weeks and 13 weeks, then dissolved in 1 ml water for injection and examined using the methods described in example 1: SEC HPLC, RP HPLC, western blot, and SDS Page (results of the examinations are presented in Table 13).

The results show that the lyophilizates of the formulations made in accordance with this invention are stable, even at higher protein concentrations up to 1 mg/ml after 13 weeks of storage at 40°C.

Example 14: /31

Long-term stability over 9 months

Lyophilizates were prepared in accordance with formulation 31 in example 11. The preparations were stored at temperatures of -20°C, 5°C, 25°C, 30°C, and 40°C for 9 months and examined by all the methods in example 1 after 3, 6, and 9 months.

No changes were detected in any of the parameters studied over the course of the storage time. At the end of the storage times at all temperatures, the preparation proved to be fully biologically active, had the full protein content, and in all purity determinations it had bands or peaks well below 1% of the intact G-CSF molecule.

The results show that the lyophilizates made in accordance with this invention possess long-term stability, even at elevated temperatures and, thus, far exceed the stability described in the prior art.

Table 14: Storage at 30°C

		3 M nate	6 M nate	9 M nate
	NFS 60 Test 80 - 125 %	entspricht	entspricht	entspricht
	OD 280	358 mg	360 mg	352 mg
(a)	SDS Page Nebenbande	< 1 %	< 1 %	< 1 %
(b)	Western Blot % Aggr. % Dimere	n.n. < 1 %	n.n. < 1 %	n.n. < 1 %

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		3 Monate	6 Monate	9 Monate
(c)	RP HPLC Produktpeak	> 99 %	> 99 %	> 99 %
(d)	SEC HPLC Produktpeak Nebenpeaks	> 98 % n.n.	> 98 % n.n.	> 98 % n.n.
(e)	Trübungsmessung TE/F	0,5	0,5	0,5

Monate=months
entspricht=corresponds
n.n.=not detectable

(a)
SDS Page
Side bands
(b)
Western blot
%aggregates
%dimers
(c)
RP HPLC
Product peak
(d)
SEC HPLC
Product peak
Side peaks

(e)
Turbidity
measurement
TE/F

Tabl 13:

(a)

Prüfparameter	Rezeptur 32						Rezeptur 33					
	0 Wo [KS]	13 Wo [-20°C]	13 Wo [KS]	13 Wo [RT]	13 Wo [30 °C]	13 Wo [40 °C]	0 Wo [KS]	13 Wo [-20°C]	13 Wo [KS]	13 Wo [RT]	13 Wo [30 °C]	13 Wo [40 °C]
Visuelle Prüfung							-	weiß	weiß	weiß	weiß	weiß
- Aussehen Lyophilisat	-	weiß	weiß	weiß	weiß	weiß	-	klar,	klar,	klar,	klar,	klar,
- Klarheit, (Lösung)	-	klar,	klar,	klar,	klar,	klar,						
SEC-HPLC												
[Reinheit %]	> 98	> 98	> 98	> 98	> 98	> 98	> 98	> 98	> 98	> 98	> 98	> 98
[Dimere/Aggregate %]	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
RP-HPLC												
[Reinheit %]	> 98	> 98	> 98	> 98	> 98	> 98	> 98	> 98	> 98	> 98	> 98	> 98
[Summe der Nebenpeaks %]	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Western-Blot [Dimere %]	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.
[Aggregate %]	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.
[Abbauprodukte %]	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.
SDS-PAGE, silver stain [Monomer %]	-	>> 99%	>> 99%	>> 99%	>> 99%	>> 99%	-	>> 99%	>> 99%	>> 99%	>> 99%	>> 99%
[Zusatzbanden %]	-	n.n.	n.n.	n.n.	n.n.	n.n.	-	n.n.	n.n.	n.n.	n.n.	n.n.
[Abbauprodukte %]	-	<< 1%	<< 1%	<< 1%	<< 1%	<< 1%	-	<< 1%	<< 1%	<< 1%	<< 1%	<< 1%

n.n. = nicht nachweisbar

Key to Table 13:
Rezeptur=Formulation
n.n=not detectable
weiß=white
klar=clear
Wo.=weeks

(a)
Visual inspection;
-appearance of lyophilizate;
-clarity (solution)
SEC HPLC;
[Purity, %];
[Dimers/aggregate, %]
RP HPLC;
[Purity, %];
[Sum of side peaks, %]
Western blot;
[Dimers, %];
[Aggregate, %];
[Decomposition products, %]
SDS Page, silver stain;
[Monomers, %];
[Additional bands, %];
[Decomposition products, %]

Claims

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1. A lyophilized pharmaceutical preparation of G-CSF, containing maltose, raffinose, sucrose, trehalose, or amino sugars.
2. A lyophilized preparation as recited in Claim 1, also containing a physiologically tolerable quantity of surfactants, which is less than or at most equal to the quantity of G-CSF protein.
3. A lyophilized preparation as recited in Claim 2, containing 0.5 mg/ml, preferably 0.01-0.1 mg/ml surfactant.

4. A lyophilized preparation as recited in one of the Claims 1 through 3, also containing a physiologically tolerable of amino acids.

5. A lyophilized preparation as recited in Claim 4, containing arginine and/or phenylalanine.

6. A lyophilized preparation as recited in one of the Claims 1 through 5, containing physiologically tolerable auxiliary agents, selected from the group of antioxidants, complexing agents, buffers, acids, bases, or isotonizing agents.

7. A lyophilized preparation as recited in one of the Claims 1 through 6, containing phosphate or acetate buffer.

8. A lyophilized preparation as recited in one of the Claims 1 through 6, containing arginine phosphate, arginine chloride, or arginine citrate buffer, preferably with a pH of 7-8.

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9. A lyophilized preparation as recited in one of the Claims 1 through 8, characterized in that it is essentially free of protein-like auxiliary agents or polymer auxiliary agents.

10. An aqueous pharmaceutical preparation that can be prepared by redissolution of the lyophilizates in accordance with Claims 1 through 9.

11. An aqueous pharmaceutical preparation as recited in Claim 10, characterized in that the solution has a pH of 6.5-8 or 3-5, preferably a pH of 7.0-7.5.

12. A process for producing a lyophilized pharmaceutical preparation in accordance with one of the Claims 1 through 9, characterized in that an aqueous preparation containing G-CSF as the active substance and maltose, raffinose, sucrose, trehalose, or amino sugar as auxiliary agents and, optionally, additional pharmaceutical auxiliary agents, is produced and the solution is then lyophilized.

13. The use of maltose, raffinose, sucrose, trehalose, or amino sugars to prepare a stable lyophilized pharmaceutical preparation contain G-CSF as the active substance.